

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 2 of 19

AMENDMENTS TO THE CLAIMS

The following Listing of the Claims will replace all prior versions and all prior listings of the claims in the present application:

Listing of The Claims:

1. (Currently Allowed) A purified thermostable DNA polymerase having an amino acid sequence presented in SEQ ID NO: 2 from residue 1 to 776.

Claims 2-4. (Previously Cancelled)

5. (Currently Allowed) An isolated recombinant polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

Claims 6-47. (Previously Cancelled)

48. (Currently Cancelled) A recombinant vector comprising a nucleic acid sequence encoding the DNA polymerase of any one of claims 1, 5, 6, 10, 16, 46 or 47.

49. (Currently Cancelled) A method of labeling a complementary strand of DNA, which method comprises the step of:

contacting a template DNA molecule with:

a recombinant Family B DNA polymerase of claim 10; and

a non-conventional nucleotide under conditions and for a time sufficient to permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate said non-conventional nucleotide into said complementary DNA strand.

50. (Currently Cancelled) A method of labeling a complementary strand of DNA, which method comprises the step of:

contacting a template DNA molecule with:

a recombinant Family B DNA polymerase comprising an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 3 of 19

corresponding to L408 or P410 or S345 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides; and

a non-conventional nucleotide under conditions and for a time sufficient to permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate said non-conventional nucleotide into said complementary DNA strand.

51. (Currently Cancelled) The method of claim 50 wherein said recombinant Family B DNA polymerase is 3'-5' exonuclease deficient.
52. (Currently Cancelled) The method of any one of claims 49-51 herein said recombinant Family B polymerase comprises a leucine to histidine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.
53. (Currently Cancelled) The method of any one of claims 49-51 wherein said recombinant Family B polymerase comprises a leucine to phenylalanine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.
54. (Currently Cancelled) The method of any one of claims 49-51 wherein said recombinant Family B polymerase comprises a proline to leucine mutation at a site corresponding to amino acid P410 of SEQ ID NO: 2.
55. (Currently Cancelled) The method of any one of claims 49-51 wherein said recombinant Family B polymerase comprises an alanine to threonine mutation at a site corresponding to amino acid A485 of SEQ ID NO: 2.
56. (Currently Cancelled) The method of claim 55 wherein said recombinant Family B polymerase comprises a leucine to histidine mutation at an amino acid corresponding to L408 of SEQ ID NO: 2.
57. (Currently Cancelled) The method of claim 55 wherein said recombinant Family B polymerase comprises a leucine to phenylalanine mutation at an amino acid corresponding to L408 of SEQ ID NO: 2.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 4 of 19

58. (Currently Cancelled) The method of claim 55 wherein said recombinant Family B polymerase comprises a proline to leucine mutation at an amino acid corresponding to P410 of SEQ ID NO: 2.
59. (Currently Cancelled) The method of claim 55 wherein said recombinant Family B polymerase comprises a serine to proline mutation at an amino acid corresponding to S345 of SEQ ID NO: 2.
60. (Currently Cancelled) The method of claim 55, wherein said recombinant Family B polymerase has a dual mutation comprising a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2; and a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.
61. (Currently Cancelled) The method of any one of claims 49-51 wherein said recombinant Family B polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides, and conjugated nucleotides.
62. (Currently Cancelled) The method of claim 61 wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.
63. (Currently Cancelled) A method of sequencing DNA comprising the steps of:
- contacting a template DNA strand with:
 - a sequencing primer;
 - a recombinant Family B DNA polymerase of claim 9; and
 - a chain-terminating nucleotide analog, under conditions that permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate nucleotides into the synthesized complementary DNA strand, wherein incorporation of a

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 5 of 19

chain-terminating nucleotide analog results in the termination of chain elongation, such that the nucleotide sequence of said DNA strand is determined.

64. (Currently Cancelled) A method of sequencing DNA comprising the steps of:

contacting a DNA strand with:

a sequencing primer;

a recombinant Family B DNA polymerase comprising an alanine to threonine mutation at a site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site corresponding to L408 or P410 or S345 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides; and

a chain-terminating nucleotide analog, under conditions that permit said DNA polymerase to synthesize a complementary DNA strand and to incorporate nucleotides into the synthesized complementary DNA strand, wherein incorporation of a chain-terminating nucleotide analog results in the termination of chain elongation, such that the nucleotide sequence of said DNA strand is determined.

65. (Currently Cancelled) The method of claim 64 wherein said recombinant Family B polymerase is deficient in 3' to 5' exonuclease activity.

66. (Currently Cancelled) The method of any one of claims 63-65 wherein said recombinant Family B polymerase has a leucine to histidine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.

67. (Currently Cancelled) The method of any one of claims 63-65 wherein said recombinant Family B polymerase has a leucine to phenylalanine mutation at a site corresponding to amino acid L408 of SEQ ID NO: 2.

68. (Currently Cancelled) The method of any one of claims 63-65 wherein said recombinant Family B polymerase has a proline to leucine mutation at a site corresponding to amino acid P410 of SEQ ID NO: 2.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 6 of 19

69. (Currently Cancelled) The method of any one of claims 63-65 wherein said recombinant Family B polymerase has an alanine to threonine mutation at a site corresponding to amino acid A485 of SEQ ID NO: 2.
70. (Currently Cancelled) The method of claim 69 wherein said recombinant Family B polymerase has a leucine to histidine mutation at a site corresponding to L408 of SEQ ID NO: 2.
71. (Currently Cancelled) The method of claim 69 wherein said recombinant Family B polymerase has a leucine to phenylalanine mutation at a site corresponding to L408 of SEQ ID NO: 2.
72. (Currently Cancelled) The method of claim 69 wherein said recombinant Family B polymerase has a proline to leucine mutation at a site corresponding to P410 of SEQ ID NO: 2.
73. (Currently Cancelled) The method of claim 69 wherein said recombinant Family B polymerase has a serine to proline mutation at a site corresponding to S345 of SEQ ID NO: 2.
74. (Currently Cancelled) The method of any one of claims 63-65 wherein said chain-terminating nucleotide analog is a dideoxynucleotide.
75. (Currently Cancelled) The method of claim 74 wherein said dideoxynucleotide is detectably labeled.
76. (Currently Cancelled) The method of claim 75 wherein said dideoxynucleotide is fluorescently labeled.
77. (Currently Cancelled) The method of claim 76 wherein said dideoxynucleotide is labeled with a moiety selected from the group consisting of fluorescein and rhodamine.
78. (Currently Cancelled) A kit for performing the method of claim 49, 50, 51, 63, or 64.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 7 of 19

79. (Currently Cancelled) A method of making a purified thermostable DNA polymerase having an amino acid sequence presented in SEQ ID NO: 2 from residue 1 to 776, comprising culturing a host cell containing the nucleic acid sequence presented in SEQ ID NO:1 under conditions which permit production of said DNA polymerase.
80. (Currently Cancelled) A method of making a recombinant DNA polymerase of *Thermococcus* species JDF-3 that is 3' to 5' exonuclease deficient, comprising culturing a host cell containing a nucleic acid sequence encoding said polymerase under conditions which permit production of said DNA polymerase.
81. (Currently Cancelled) A method of making a recombinant DNA polymerase having reduced discrimination against non-conventional nucleotides, comprising culturing a host cell containing a nucleic acid sequence encoding said polymerase under conditions which permit production of said DNA polymerase.
82. (Currently Cancelled) A method of making a recombinant Family B DNA polymerase comprising an alanine to threonine mutation at the site corresponding to A485 of SEQ ID NO: 2 or a mutation at a site corresponding to L408 or P410 or S345 of SEQ ID NO: 2, wherein said DNA polymerase has reduced discrimination against non-conventional nucleotides relative to the wild-type form of that polymerase, comprising culturing a host cell containing a nucleic acid sequence encoding said polymerase under conditions which permit production of said DNA polymerase.
83. (Currently Cancelled) The method of claim 79, 80, 81, or 82, wherein said host cell is *E. Coli*.
84. (Currently Cancelled) The method of claim 79, 80, 81, or 82, wherein said host cell is *Thermococcus*.

Claims 85-88 are Previously Cancelled.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 8 of 19

89. (Currently Cancelled) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence of SEQ ID NO: 2.

90. (Currently Cancelled) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence of SEQ ID NO: 2 and wherein said mutation in the exo I (DXE) motif is selected from the group consisting of: aspartic acid (D) to threonine (T), aspartic acid (D) to alanine (A) and glutamic acid (E) to alanine (A).

91. (Currently Cancelled) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence of SEQ ID NO: 2 and a mutation at one or more amino acids in Region II (DXXSLYPSII) within said sequence of SEQ ID NO: 2.

92. (Currently Cancelled) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence of SEQ ID NO: 2 and a mutation at one or more amino acids in Region II (DXXSLYPSII) within said sequence of SEQ ID NO: 2, wherein said mutation in the exo I (DXE) motif is selected from the group consisting of: aspartic acid (D) to threonine (T), aspartic acid (D) to alanine (A) and glutamic acid (E) to alanine (A), and said mutation in Region II (DXXSLYPSII) is selected from the group consisting of: leucine (L) to histidine (H), leucine (L) to phenylalanine (F), and proline (P) to leucine (L).

93. (Currently Cancelled) The isolated recombinant DNA polymerase of any one of claims 89-92, further comprising a mutation at one or more additional amino acids selected from the group consisting of: S345, A485, T604, Y497, I630, E645, E578, R465, V401, N424, P569, E617, V640, S651, L396, E459, L456, E658, V437, L478, Y496, Y409 and A490 within the sequence of SEQ ID NO: 2.

94. (Currently Cancelled) The isolated recombinant polymerase of claim 93, wherein said mutation at S345 is serine (S) to proline (P), said mutation at A485 is alanine (A) to

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 9 of 19

threonine (T), cysteine (C), serine (S), leucine (L), isoleucine (I), phenylalanine(F) or valine (V), said mutation at Y497 is tyrosine (Y) to cysteine (C), said mutation at I630 is isoleucine (I) to valine (V), said mutation at E645 is glutamic acid (E) to lysine (L), said mutation at E578 is glutamic acid (E) to lysine (L), said mutation at R465 is arginine (R) to methionine (M), said mutation at L396 is leucine (L) to glutamine (Q) or proline (P), said mutation at S651 is serine (S) to asparagine (B), said mutation at L456 is leucine (L) to histidine (H), said mutation at Y496 is tyrosine (Y) to asparagine (B) or leucine (L), said mutation at Y409 is tyrosine (Y) to valine (V), said mutation at A490 is alanine (A) to tyrosine (Y).

95. (Currently Cancelled) The isolated recombinant DNA polymerase of any one of claims 89-91, wherein said isolated recombinant DNA polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

96. (Currently Cancelled) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in the exo II (NX₂₋₃FD) motif or the exo III (YX₃D) within said sequence of SEQ ID NO: 2.

97. (Currently Cancelled) An isolated recombinant DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at one or more amino acids in the exo II (NX₂₋₃FD) motif or the exo III (YX₃D) motif within said sequence of SEQ ID NO: 2 and a mutation at one or more amino acids in Region II (DXXSLYPSII) within said sequence of SEQ ID NO: 2.

98. (Currently Cancelled) The isolated recombinant DNA polymerase of claim 96 or 97, wherein said isolated recombinant DNA polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

99. (Currently Cancelled) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 10 of 19

listed in Table II, and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

100. (Currently Cancelled) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences listed in Table II as indicated by accession number, and further comprising a mutation at one or more amino acids in the exo II (NX₂₋₃FD) motif or the exo III (YX₃D) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

101. (Currently Cancelled) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence, a mutation in Region II (DXXSLYPSII) within said sequence, and a mutation at amino acid corresponding to A485 of SEQ ID NO: 2.

102. (Currently Cancelled) An isolated recombinant family B DNA polymerase comprising a sequence selected from the sequences listed in Table II as indicated by accession number, and further comprising a mutation at one or more amino acids in the exo II (NX₂₋₃FD) motif or the exo III (YX₃D) motif within said sequence, a mutation in Region II (DXXSLYPSII) within said sequence, and a mutation at amino acid corresponding to A485 of SEQ ID NO: 2.

103. (Currently Cancelled) The isolated recombinant family B DNA polymerase of claim 99 or 100, further having reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

104. (Currently Cancelled) An isolated recombinant thermostable family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in the exo I (DXE) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 11 of 19

105. (Currently Cancelled) An isolated recombinant thermostable family B DNA polymerase comprising a sequence selected from the sequences as indicated by accession numbers listed in Table II, and further comprising a mutation at one or more amino acids in the exo II (NX₂₋₃FD) motif or the exo III (YX₃D) motif within said sequence and a mutation at the leucine and/or proline positions in Region II (DXXSLYPSII) within said sequence.

106. (Currently Cancelled) The isolated recombinant thermostable family B DNA polymerase of claim 104 or 105, further having reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

107. (Currently Cancelled) The isolated recombinant thermostable family B DNA polymerase of claim 104 or 105, wherein said recombinant thermostable family B DNA polymerase is an archaeal DNA polymerase.

108. (Currently Allowed) An isolated recombinant JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at D141 and/or E143 within said sequence of SEQ ID NO: 2.

109. (Currently Allowed) The isolated recombinant JDF-3 DNA polymerase of claim 108, wherein said mutation at D141 is an aspartic acid (D) to threonine (T) or alanine (A) mutation, and said mutation at E143 is a glutamic acid (E) to alanine (A) mutation.

110. (Currently Cancelled) The isolated recombinant JDF-3 DNA polymerase of any one of claims 108-109, further comprising a mutation at one or more amino acids of Region II (DXXSLYPSII) within said SEQ ID NO: 2.

111. (Currently Amended) The isolated recombinant JDF-3 DNA polymerase of claim [110]108 or 109, [wherein said] further comprising a mutation at [one or more amino acids is at] L408 and/or P410[within Region II].

112. (Currently Amended) The isolated recombinant JDF-3 DNA polymerase of claim 111, wherein said mutation at [one or more amino acids]L408 is [selected from the group

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 12 of 19

consisting of:] a leucine (L) to histidine (H) or phenylalanine (F) mutation[at L408] and said mutation at P410 is a proline (P) to leucine ([P]L) mutation[at P410].

113. (Currently Amended) The isolated recombinant JDF-3 DNA polymerase of [any one of claims 108-109]claim 108 or 109, further comprising a mutation at one or more additional amino acids selected from the group consisting of: A485, S345, T604, Y497, I630, E645, E578, R465, V401, N424, P569, E617, V640, S651, L396, E459, L456, E658, V437, L478, Y496, Y409 and [Y490]A490 within the sequence of SEQ ID NO: 2.

114. (Currently Amended) The isolated recombinant [LDF-3]JDF-3 DNA polymerase of claim [108]113, wherein said mutation at S345 is serine (S) to proline (P), said mutation at A485 is alanine (A) to threonine (T), cysteine (C), serine (S), leucine (L), isoleucine (I), phenylalanine(F) or valine (V), said mutation at Y497 is tyrosine (Y) to cysteine (C), said mutation at I630 is isoleucine (I) to valine (V), said mutation at E645 is glutamic acid (E) to lysine (L), said mutation at E578 is glutamic acid (E) to lysine (L), said mutation at R465 is arginine (R) to methionine (M), said mutation at L396 is leucine (L) to glutamine (Q) or to proline (P), said mutation at S651 is serine (S) to asparagine (B), said mutation at L456 is leucine (L) to histidine (H), said mutation at Y496 is tyrosine (Y) to asparagine (B) or leucine (L), said mutation at Y409 is tyrosine (Y) to valine (V), said mutation at [Y490]A490 is alanine (A) to tyrosine (Y).

115. (Currently Amended) The isolated recombinant JDF-3 DNA polymerase of [any one of claims 108-109]claim 108 or 109, wherein said JDF-3 DNA polymerase has reduced discrimination against a non-conventional nucleotide selected from the group consisting of: dideoxynucleotides, ribonucleotides and conjugated nucleotides.

116. (Currently Allowed) The isolated recombinant JDF-3 DNA polymerase of claim 115, wherein said conjugated nucleotide is selected from the group consisting of radiolabeled nucleotides, fluorescently labeled nucleotides, biotin labeled nucleotides, chemiluminescently labeled nucleotides and quantum dot labeled nucleotides.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 13 of 19

117. (Currently Amended) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations: D141T or D141A, E143A, L408H or L408F, P410L, and A485T within said [sequence]SEQ ID NO: 2.

118. (Currently Amended) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations: D141T or D141A and E143A within said [sequence]SEQ ID NO: 2.

119. (Currently Amended) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations: D141T or D141A and E143A, and further comprising one or more mutations selected from the group consisting of: L408H or L408F, P410L, and S345P within said [sequence]SEQ ID NO: 2.

120. (Currently Amended) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising mutations at: D141, E143, P410, and A485 within said [sequence]SEQ ID NO: 2.

121. (Currently Amended) An isolated JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising the following mutations of: D141T or D141A, E143A, P410L, and A485T within said [sequence]SEQ ID NO: 2.

122. (Currently Amended) A kit comprising an isolated recombinant polypeptide [DNA polymerase of any one of claims 89-92] of claim 5, and packaging material thereof.

123. (Currently Amended) A kit comprising an isolated recombinant DNA polymerase of [any one of claims 96-97] claim 108 or 109, and packaging material thereof.

124. (Currently Amended) A kit comprising an isolated recombinant DNA polymerase of [any one of claims 99-102] 111, and packaging material thereof.

125. (Currently Amended) A kit comprising an isolated recombinant DNA polymerase of [any one of claims 104-105] claim 112, and packaging material thereof.

126. (Currently Amended) A kit comprising an isolated recombinant DNA polymerase of [any one of claims 108, 117-119] claim 113, and packaging material thereof.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 14 of 19

126. (Currently Amended) A kit comprising an isolated recombinant DNA polymerase of [any one of claims 108, 117-119]claim 113, and packaging material thereof.

127. (Currently Amended) A kit comprising an isolated recombinant DNA polymerase of [any one of claims 120-121]claim 114, and packaging material thereof.

128. (Currently Cancelled) The isolated JDF-3 DNA polymerase of claim 117, further comprising both mutations P410L and A485T.

129. (Currently Cancelled) The isolated JDF-3 DNA polymerase of claim 117, further comprising both mutations D141A and E143A.

130. (Currently Cancelled) A kit comprising an isolated DNA polymerase of claim 128 or 129, and packaging material thereof.

131. (Currently Cancelled) A method of synthesizing a complementary strand of DNA, said method comprising:

contacting a template DNA molecule with a non-conventional nucleotide and a recombinant JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at D141 and/or E143 within said sequence of SEQ ID NO: 2; and

incorporating said non-conventional nucleotide to synthesize a complementary DNA strand.

132. (Currently Cancelled) The method of claim 131, wherein said recombinant JDF-3 DNA polymerase further comprises a mutation at L408 and/or P410.

133. (Currently Cancelled) The method of claim 132, wherein said mutation at D141 is an aspartic acid (D) to threonine (T) or alanine (A) mutation, said mutation at E143 is a glutamic acid (E) to alanine (A) mutation, said mutation at L408 is a leucine (L) to histidine (H) or phenylalanine (F) mutation, and said mutation at P410 is a proline (P) to leucine (L) mutation.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 15 of 19

134. (Currently Cancelled) The method of claim 131, 132, or 133, wherein said recombinant JDF-3 DNA polymerase further comprises a mutation at one or more additional amino acids selected from the group consisting of: A485, S345, T604, Y497, I630, E645, E578, R465, V401, N424, P569, E617, V640, S651, L396, E459, L456, E658, V437, L478, Y496, Y409 and A490 within the sequence of SEQ ID NO: 2.

135. (Currently Cancelled) The method of claim 134, wherein said mutation at S345 is serine (S) to proline (P), said mutation at A485 is alanine (A) to threonine (T), cysteine (C), serine (S), leucine (L), isoleucine (I), phenylalanine (F) or valine (V), said mutation at Y497 is tyrosine (Y) to cysteine (C), said mutation at I630 is isoleucine (I) to valine (V), said mutation at E645 is glutamic acid (E) to lysine (L), said mutation at E578 is glutamic acid (E) to lysine (L), said mutation at R465 is arginine (R) to methionine (M), said mutation at L396 is leucine (L) to glutamine (Q) or to proline (P), said mutation at S651 is serine (S) to asparagine (B), said mutation at L456 is leucine (L) to histidine (H), said mutation at Y496 is tyrosine (Y) to asparagine (B) or leucine (L), said mutation at Y409 is tyrosine (Y) to valine (V), said mutation at A490 is alanine (A) to tyrosine (Y).

136. (Currently Cancelled) A method of sequencing DNA comprising the steps of:

contacting a template DNA strand with a sequencing primer, a chain-terminating nucleotide analog, and a recombinant JDF-3 DNA polymerase comprising a sequence of SEQ ID NO: 2 and further comprising a mutation at D141 and/or E143 within said sequence of SEQ ID NO: 2; and

incorporating said non-conventional nucleotide to synthesize a complementary DNA strand, wherein incorporation of said chain-terminating nucleotide analog results in the termination of chain elongation, such that the nucleotide sequence of said DNA strand is determined.

137. (Currently Cancelled) The method of claim 136, wherein said recombinant JDF-3 DNA polymerase further comprises a mutation at L408 and/or P410.

138. (Currently Cancelled) The method of claim 137, wherein said mutation at D141 is an aspartic acid (D) to threonine (T) or alanine (A) mutation, said mutation at E143 is a

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 16 of 19

glutamic acid (E) to alanine (A) mutation, said mutation at L408 is a leucine (L) to histidine (H) or phenylalanine (F) mutation, and said mutation at P410 is a proline (P) to leucine (L) mutation.

139. (Currently Cancelled) The method of claim 136, 137, or 138, wherein said recombinant JDF-3 DNA polymerase further comprises a mutation at one or more additional amino acids selected from the group consisting of: A485, S345, T604, Y497, I630, E645, E578, R465, V401, N424, P569, E617, V640, S651, L396, E459, L456, E658, V437, L478, Y496, Y409 and A490 within the sequence of SEQ ID NO: 2.

140. (Currently Cancelled) The method of claim 139, wherein said mutation at S345 is serine (S) to proline (P), said mutation at A485 is alanine (A) to threonine (T), cysteine (C), serine (S), leucine (L), isoleucine (I), phenylalanine (F) or valine (V), said mutation at Y497 is tyrosine (Y) to cysteine (C), said mutation at I630 is isoleucine (I) to valine (V), said mutation at E645 is glutamic acid (E) to lysine (L), said mutation at E578 is glutamic acid (E) to lysine (L), said mutation at R465 is arginine (R) to methionine (M), said mutation at L396 is leucine (L) to glutamine (Q) or to proline (P), said mutation at S651 is serine (S) to asparagine (B), said mutation at L456 is leucine (L) to histidine (H), said mutation at Y496 is tyrosine (Y) to asparagine (B) or leucine (L), said mutation at Y409 is tyrosine (Y) to valine (V), said mutation at A490 is alanine (A) to tyrosine (Y).

141. (Currently Cancelled) The method of claim 136, wherein said chain-terminating nucleotide analog is a dideoxynucleotide.

142. (Currently Cancelled) The method of claim 141 wherein said dideoxynucleotide is detectably labeled.

143. (Currently Cancelled) The method of claim 142, wherein said dideoxynucleotide is fluorescently labeled.

144. (Currently Cancelled) The method of claim 143, wherein said dideoxynucleotide is labeled with a moiety selected from the group consisting of fluorescein and rhodamine.

Application Serial Number 09/698,341
Attorney Docket No. 25436/1560
Page 17 of 19

145. (Previously Added and Currently Amended) A method of making [an isolated recombinant JDF-3]a purified thermostable DNA polymerase of claim 1, comprising a) transfecting a host cell with[culturing a host cell containing] the nucleic acid sequence presented in SEQ ID NO:1, and b) culturing said host cell under conditions which permit production of said DNA polymerase.

146. (Previously Added) The method of claim 145, wherein said host cell is *E. Coli* or *Thermococcus*.

147. (Previously Added and Currently Amended) A method of making [an isolated recombinant JDF-3]a purified thermostable DNA polymerase of claim 108 or 109, comprising a) transfecting a host cell[culturing a host cell containing]with a nucleic acid sequence encoding said polymerase and b) culturing said host cell under conditions which permit production of said DNA polymerase.

148. (Previously Added) The method of claim 147, wherein said host cell is *E. Coli* or *Thermococcus*.